

APPENDIX A

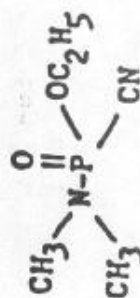
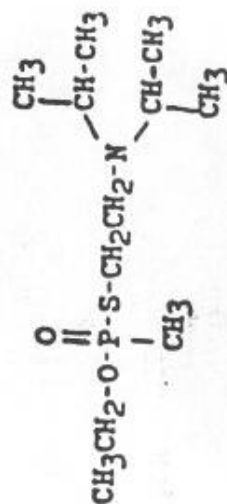
MASTER FILE--ANTICHOLINESTERASE CHEMICALS^a

Tox Num A001
EA Num EA 1208
Military Desig GB
Chemical Name Isopropylmethylphosphonofluoridate
CAS Num 107-44-8
Common Name Sarin
Chemical Type Organophosphorus ester

Tox Num A002
EA Num EA 1701
Military Desig VX
Chemical Name Ethyl S-2-diisopropylaminoethyl methylphosphonothiolate
CAS Num 5-782-69-9
Chemical Type Organophosphorus ester

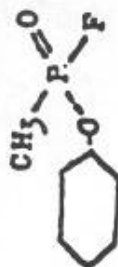
Tox Num A003
EA Num EA 1205
Military Desig GA
Chemical Name Ethyl N,N-dimethylphosphoramidocyanate
CAS Num 77-81-6
Common Name Tabun
Common Name Taboon A
Common Name Trilon 83
Common Name Gelan I
Other Num MCE
Chemical Type Organophosphorus ester

Structural Formula

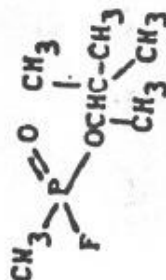


^aTox Num = arbitrary NRC designation; EA Num = Edgewood Arsenal designation.

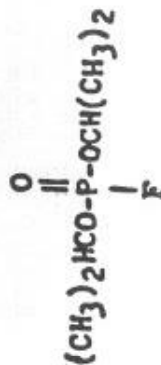
Tox Num A004
 EA Num EA 1212
 Military Desig GP
 Chemical Name Cyclohexyl methylphosphonofluoridate
 CAS Num 329-99-7
 Chemical Type Organophosphorus ester



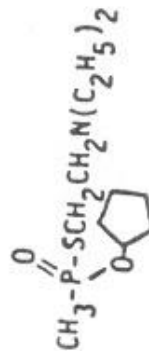
Tox Num A005
 EA Num EA 1210
 Military Desig GD
 Chemical Name Pinacolyl methylphosphonofluoridate
 CAS Num 96-64-0
 Common Name Soman
 Other Num T.2107
 Chemical Type Organophosphorus ester



Tox Num A006
 Abbreviated Name - PF3
 EA Num EA 1152
 Military Desig DFP
 Chemical Name Diisopropyl fluorophosphate
 CAS Num 55-91-4
 Other Num T.1703
 Chemical Type Organophosphorus ester



Tox Num A007
 EA Num EA 3148
 Chemical Name Cyclopentyl S-2-diethylaminoethyl methylphosphonothiolate
 Chemical Type Organophosphorus ester



Tox Num A008
 EA Num EA 1285
 Chemical Name Tetraethyl pyrophosphate
 CAS Num 107-49-3
 Common Name TEPP
 Chemical Type Organophosphorus ester



Tox Num A009

Abbreviated Name - THA

Chemical Name 9-Amino-1,2,3,4-tetrahydroacridine

CS Num CS 12602

CAS Num 321-64-2

Common Name Tacrine

Common Name Romotal

Chemical Type Acridine

Tox Num A010

Chemical Name Physostigmine

CS Num CS 58525

CAS Num 57-47-6 (free base)

CAS Num 64-47-1 (sulfate)

CAS Num 57-64-7 (salicylate)

Common Name Eserine

Chemical Type Carbamate

Tox Num A011

Chemical Name m-(Hydroxyphenyl)trimethylammonium methylsulfate dimethylcarbamate

CAS Num 51-60-5 (methyl sulfate)

CAS Num 114-80-7 (bromide)

CAS Num 59-99-4

Common Name Prostigmin

Common Name Neostigmine methyl sulfate

Chemical Type Carbamate

Tox Num A012

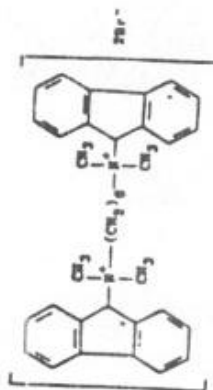
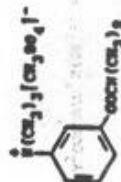
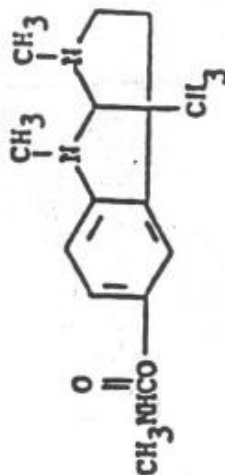
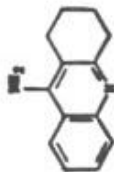
Chemical Name Hexamethylenebis[9-fluorenyldimethylammonium bromide]

CAS Num 317-52-2

Common Name Mylaxen

Common Name Hexafluorenum bromide

Chemical Type Curare



Tox Num A013

Chemical Name 3-Hydroxy-1-methylpyridinium bromide dimethylcarbamate

CAS Num 155-97-5

Common Name Pyridostigmine bromide

Common Name Mestinon

Chemical Type Carbamate

Tox Num A014

Chemical Name S-[1,2-bis(ethoxycarbonyl)ethyl]-O,O-dimethyl phosphorodithioate

CAS Num 121-75-5

Common Name Malathion

Chemical Type Organophosphorus ester

Tox Num A020

Pharm Class Direct cholinergic

Chemical Name (2-Hydroxypropyl)trimethylammonium chloride acetate

CAS Num 62-51-1

Common Name Mecholyl chloride

Common Name Methacholine

Chemical Type Choline ester

Tox Num A021

Pharm Class Direct cholinergic

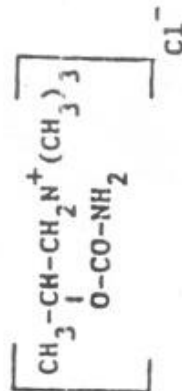
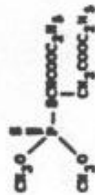
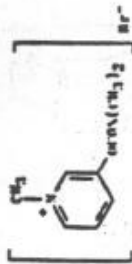
Chemical Name (2-Hydroxypropyl)trimethylammonium chloride carbamate

CAS Num 590-63-6

Common Name Urecholine chloride

Common Name Bethanecol chloride

Chemical Type Quaternary ammonium carbamate

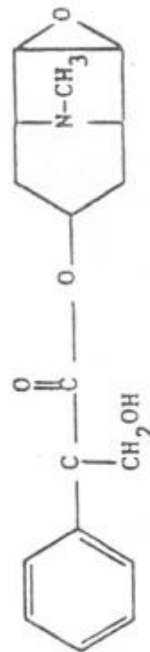
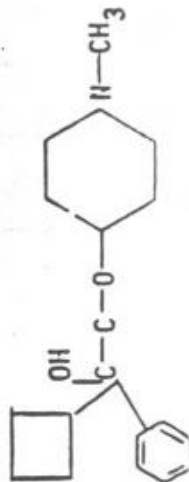
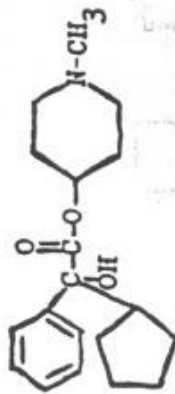
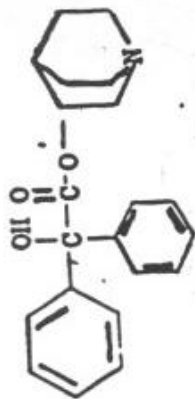


APPENDIX B

MASTER FILE--ANTICHOLINERGIC CHEMICALS^a

Structural Formula

Tox Num: B001	
Abbreviated Name: QNB	
EA Num: EA 2277	
Military Design: BZ	
Chemical Name: 3-Quinuclidinyl benzilate	
CS Num: CS 4030	
CAS Num: 13004-56-3 (hydrochloride)	
CAS Num: 6581-06-2 (free base)	
CAS Num: 24572-08-5 (hydrobromide)	
Other Num: R02-3308	
Chemical Type: Glycolic acid ester	
Tox Num: B002	
EA Num: EA 3443	
Chemical Name: N-Methyl-4-piperidyl cyclopentylphenylglycolate	
CAS Num: 37830-21-0	
Chemical Type: Glycolic acid ester	
Tox Num: B003	
EA Num: EA 3580	
Chemical Name: N-Methyl-4-piperidyl cyclobutylphenylglycolate	
CAS Num: 54390-94-2	
Chemical Type: Glycolic acid ester	
Tox Num: B004	
Cont Num: 300113	
CAS Num: 51-34-3 (free base)	
CAS Num: 55-16-3 (hydrochloride)	
Common Name: scopolamine	
Common Name: Hyoscine	
Common Name: Sominex	
Chemical Type: Glycolic acid ester	



^aTox Num arbitrary NRC designation; EA Num Edgewood Arsenal designation; Cont Num contractor's designation.

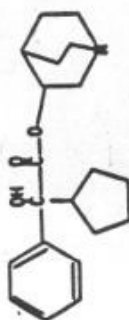
Tox Num: B005

CAS Num: 51-55-8 (free base)
 CAS Num: 55-48-1 (sulfate)
 CAS Num: 33952-38-4 (hydrochloride)
 Common Name: Atropine
 Chemical Type: Glycolic acid ester



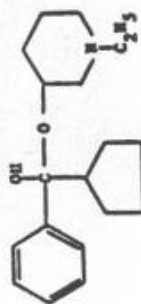
Tox Num: B006

EA Num: EA 3167
 Chemical Name: 3-Quinuclidinyl phenylcyclopentylglycolate
 CAS Num: 26758-53-2
 CAS Num: 29125-55-1 (hydrochloride)
 Chemical Type: Glycolic acid ester



Tox Num: B007

Chemical Name: 1-Ethyl-3-piperidyl α -cyclopentyl- α -phenylglycolate hydrochloride
 CS Num: CS 4297 (free base)
 CS Num: CS 62025 (hydrochloride)
 CS Num: CS 4298 (hydrochloride)
 CAS Num: 8015-54-1

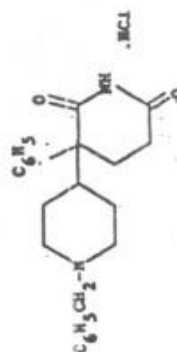


Common Name: Ditrin

Other Num: JB 329 (free base)
 Other Num: JB 840 (hydrochloride)
 Chemical Type: Glycolic acid ester

Tox Num: B008

Chemical Name: dl-2-(1-Benzyl-4-piperidyl)-2-Phenylglutarimide hydrochloride



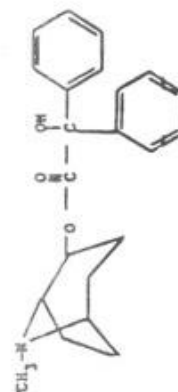
CAS Num: 14051-33-3

Common Name: Benzetimide hydrochloride
 Common Name: Dioxatrine
 Other Num: R4929
 Other Num: MCN-JR4929
 Other Num: 4929

Chemical Type: Substituted glutarimide

Tox Num: B009

Chemical Name: L-2- α -Tropinyl benzilate hydrochloride
 CS Num: CS 27349
 Cont Num: 219758
 CAS Num: 64520-33-8
 CAS Num: 64471-12-1 (free base)
 Chemical Type: Glycolic acid ester



Tox Num: B010

Chemical Name: L-2- α -Tropinyl L-cyclopentylphenylglycolate

Cont Num: 226086

CAS Num: 64471-85-8

Chemical Type: Glycolic acid ester

Tox Num: B011

Chemical Name: N-Methyl-4-piperidyl cyclopentyl-(1-propynyl)-glycolate

Cont Num: 302196

CAS Num: 53034-67-6

Chemical Type: Glycolic acid ester

Tox Num: B012

Chemical Name: cis-2-Methyl-3-quinuclidinyl cyclopentylphenylglycolate

Cont Num: 301060

Chemical Type: Glycolic acid ester

Tox Num: B013

Chemical Name: 1-Methyl-4-piperidyl phenyl-(3-methylbut-1-yn-3-enyl)-glycolate

Chemical Type: Glycolic acid ester

Cont Num: 302282

Tox Num: B014

Chemical Name: 3-Quinuclidinyl (1-hydroxycyclopentyl) phenylacetate

Cont Num: 302368

Chemical Type: Glycolic acid ester

Tox Num: B015

Chemical Name: 3-Quinuclidinyl cyclopentyl-(2-propenyl)-glycolate

Cont Num: 302537

Chemical Type: Glycolic acid ester

Tox Num: B016

Chemical Name: 4-(1-methyl-1,2,3,6-tetrahydropyridyl)-Methyl-Isopropylphenylglycolate

Cont Num: 302668

Chemical Type: Glycolic acid ester

Tox Num: B017

Chemical Name: 3-Quinuclidyl cyclopentyl-(1-propynyl)-glycolate

Cont Num: 302212

Chemical Type: Glycolic acid ester

Tox Num: B018

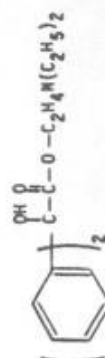
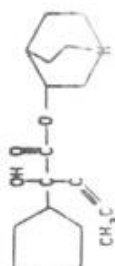
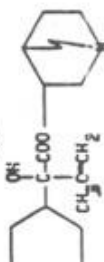
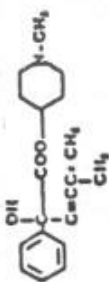
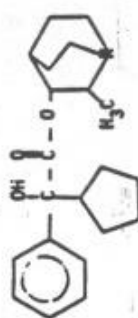
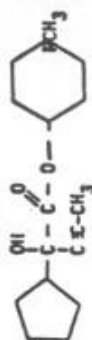
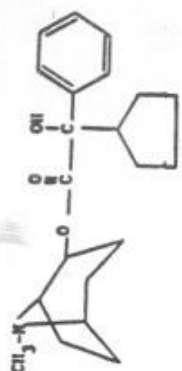
EA Num: EA 2092

Chemical Name: 2-Diethylaminoethyl diphenylglycolate

CAS Num: 57-37-4

Common Name: Benactyzine (hydrochloride)

Chemical Type: Glycolic acid ester



Tox Num: B019

Abbreviated Names: BAT; BETE; BTE

Chemical Name: Tropine benzoate

CAS Num: 3736-36-5

Chemical Type: Glycolic acid ester

Tox Num: B020

Pharm Class: Anticholinergic

Chemical Name: D-Tropine tropate

CAS Num: 55-47-0 (hydrochloride)

CAS Num: 50700-39-5 (hydrobromide)

Common Name: D-Hyoscyamine

Chemical Type: Glycolic acid ester

Tox Num: B021

CAS Num: 155-41-9

CAS Num: 6106-46-3 (nitrate)

Common Name: Methyl scopolamine

Common Name: Methscopolamine bromide

Common Name: Pamine (bromide)

Chemical Type: Glycolic acid ester

Tox Num: B022

CS Num: 60450

CAS Num: 52-88-0

Common Name: Atropine methyl nitrate

Common Name: Eumydrin

Chemical Type: Glycolic acid ester

Tox Num: B023

EA Num: EA 3834

Chemical Name: N-Methyl-4-piperidyl isopropylphenylglycolate

Chemical Type: Glycolic acid ester

Tox Num: B024

Chemical Name: 2-Diethylaminoethyl 1-phenylcyclopentanecarboxylate hydrochloride

CAS Num: 125-85-9

Common Name: Caramephen hydrochloride

Common Name: Panparnit

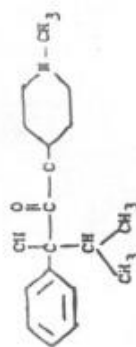
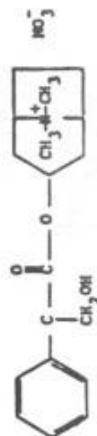
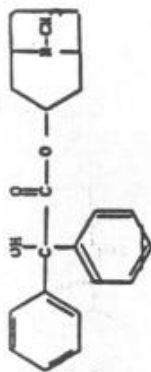
Chemical Type: Basic ester

Tox Num: B025

Abbreviated Name: TAB

Common Name: Mixture of TMB4 (D4), atropine (B5), and benactyzine (B18)

Chemical Type: Glycolic acid ester



Tox Num: B026

Chemical Name: 1-(3-Hydroxy-5-methyl-4-phenylhexyl)-1-methylpiperidinium bromide

CAS Num: 520-20-7

Common Name: Darstine

Common Name: Mepiperphenidol

Chemical Type: Piperidinium halide

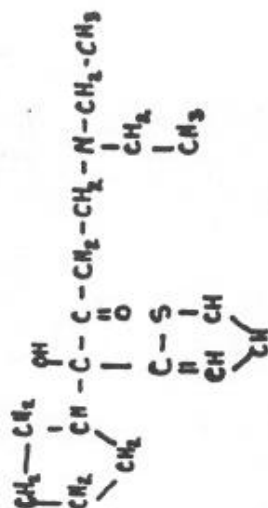
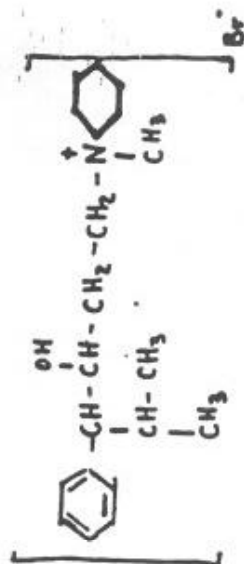
Tox Num: B027

Chemical Name: -Cyclopentyl- -hydroxy-2-thiopheneacetic acid, 2-diethylaminoethyl ester hydrochloride

CAS Num: 3737-35-7

Other Num: WIN 2299

Chemical Type: Glycolic acid ester



APPENDIX C

LIST OF DOCUMENTS SUPPLIED TO ANTICHOLINESTERASE PANEL

Background on volunteer testing at Edgewood (use of volunteers in chemical agent research)

Master file and literature references, anticholinesterase chemicals

Research and experimental case file on volunteers (case report summaries, high dose and random samples)

Anticholinesterase chemicals: digest-report

Anticholinesterase chemicals: Karczmar publication

LSD and Himsworth reports (examples of prior long-term toxicity evaluations)

Human test protocols, Edgewood volunteer program

Representative test protocols, random-sample case report summaries

Elements of final report, submitted by Panel members, dated January 21, 1982

Complete list of volunteers (anticholinesterase chemicals) with volunteer numbers (not names), agents, doses, and dates of exposure identified: contains master file and reference file of Edgewood reports

Mortality data evaluation

APPENDIX D

LIST OF DOCUMENTS SUPPLIED TO ANTICHOLINERGIC PANEL

Background on volunteer testing at Edgewood (use of volunteers in chemical agent research)

Master file and literature references, anticholinergic chemicals

Research and experimental case files on volunteers (case report summaries, high dose samples)

Anticholinergic chemicals: digest-report

Anticholinesterase chemicals: Karczmar publication

LSD and Himsworth reports (examples of prior long-term toxicity evaluations)

Human test protocols, Edgewood volunteer program

Representative test protocols, summary of digest-report investigation of two cases of reported flashback

Elements of final report, submitted by Panel members, dated January 15, 1982

Complete list of volunteers (anticholinergic chemicals) with volunteer numbers (not names), agents, doses, and dates of exposure identified: contains master file and reference file of Edgewood reports

Mortality data evaluation

APPENDIX E

CASE-FILE DATA ON EDGEWOOD SUBJECTS*

SUMMARY OF VOLUNTEER TESTING WITH GB (A1)

A total of 246 subjects was tested with GB under various conditions. Twenty-five subjects were selected for review, representing approximately 10% of the test group. Selections were based upon the following criteria:

No Treatment (9 subjects)
Route: Intravenous (3.0 - 4.0 µg/kg) 9 subjects
No symptoms
Treatment (PAMCL) (9 subjects)
Dizziness, frontal headache, blurred vision lethargy, nausea, stomach pain, vomiting.
Treatment (7 subjects)
Route: Intravenous (3.0 - 4.0 µg/kg) (P₂S) 2 subjects
Intravenous (3.0 - 4.0 µg/kg) (TMB₄) 3 subjects
Whole-body exposure to vapor (Atropine) 1 subject
Whole-body exposure to vapor (Pyrbenzamine) 1 subject
Rhinoorrhea, chest tightness, wheezing.

SUMMARY OF VOLUNTEER TESTING WITH VX (A2)

A total of 740 subjects was tested under various conditions with VX. Seventy-five subjects were selected for review, representing 10% of the test group. Selections were based upon the following criteria:

No Treatment (52 subjects)
Route: Oral (single dose; 3.5 µg/kg) 1 subject
Percutaneous (single dose; 5 - 45 µg/kg) 22 subjects
Oral (multiple dose) 29 subjects
Treatment (23 subjects 2-PAM, Atropine, Regitine)
Route: Intravenous (1.2 - 2.0 µg/kg) 7 subjects
Intramuscular 3 subjects
Oral (4.25 - 4.5 µg/kg) 3 subjects
Percutaneous (5.0 - 45 µg/kg) 10 subjects

The vast majority of these subjects reportedly experienced little or no effect from the drug. In 2 cases, the cholinesterase (RBC) level was less than 20% of normal in subjects that received multiple doses, but in each case the dosing was stopped and the cholinesterase levels returned to normal limits within 6-7 days post exposure. No visible effects of the drug were observed in any of these subjects. One subject (A2(BN)) received 30 µg/kg, percutaneously, showed some effects (headache, nausea, ChE 15% of normal, nervous) and was treated with multiple doses of atropine and 2-PAM. Subject recovered and RBCChE was 97% of normal when discharged.

Another subject (A2(BA)) received 1.5 µg/kg intravenously, developed twitching (fasciculation?) of leg muscles, perspiration of palms and soles of feet 15 min after injection when RBC ChE was 87%

*This represents selection of high-dose subjects. A second set of records of equal number was also procured, on the basis of random selection.

of normal (dropping to 17% at 1h). By 30 min, hands and feet were cold and the eyes felt "heavy." A third subject (A2(BS)), who received 3.2 µg/kg intramuscularly, was dizzy, nauseated, and very drowsy after 2h. During the next 10 hours, the subject lacked concentration and stared vacantly into space. Slight dizziness and blurred vision persisted at 26h when he was treated with 2-PAM and recovered.

Some others reportedly experienced dizziness, light-headedness, nausea, vomiting, "stiff jaw," headache, and ptosis lasting for at least 24h after exposure.

SUMMARY OF VOLUNTEER TESTING WITH GA (A3)

A total of 26 subjects was tested under various conditions with GA. Thirteen (13) subjects were selected for review, representing 50% of the test group. Selections were based upon the following criteria:

	<u>Treatment (10 subjects)</u>
Route:	Intravenous (2.0 - 3.0 µg/kg)+(PAMCl, Pretreatment) 3 subjects
	Intravenous (1.0 - 2.0 µg/kg)+(PAMCl, Post Treatment) 2 subjects
	Intravenous (3.0 µg/kg)+(P ₂ S, Pretreatment) 1 subject
	Intravenous (3.0 µg/kg)+(P ₂ S, Post Treatment) 2 subjects
	Intravenous (3.0 µg/kg)+(PAMCl+TMB ₄ , Pretreatment) 1 subject
	Intravenous (3.0 µg/kg)+(PAMCl+P ₂ S, Pretreatment) 1 subject

All subjects responded to pre- or post-exposure treatment with PAMCl or P₂S when exposed to GF. ChE levels recovered after initial depression within 24 hours or less; they were approaching normal levels by 1 to 2 days post-exposure. Treatment with PAMCl and P₂S or PAMCl and TMB₄ did not alter or significantly improve the recovery. None of these subjects reportedly had any serious or prolonged effects from the drug.

SUMMARY OF VOLUNTEER TESTING WITH GD (A5)

A total of 83 subjects was tested under various conditions with GD. Ten (10) subjects were selected for review, representing approximately 12% of the test subjects. Selections were based upon the following criteria:

	<u>No Treatment (4 subjects)</u>
Route:	Percutaneous (8.5 - 90 µg/kg) 4 subjects
	<u>Treatment (6 subjects) Atropine, PAMCl, P₂S, TMB-4</u>
Route:	Intravenous (1.0 - 2.0 µg/kg) 6 subjects

Blood cholinesterase measurements were made on the 4 subjects that received GD by the percutaneous route. None of these subjects showed any significant decrease in their ChE levels over a 2-week period. No complaints or severe effects reportedly were observed in any of the subjects tested by this route at the doses used. Two of the subjects who received GD by the intravenous route, although treated with oxime and/or atropine showed significant decrease in the ChE levels, and exhibited the "usual physiological responses" from this agent. Weakness and muscle contractions were also

experienced. They also developed persistent vomiting and nausea to a point of dehydration. One subject was referred to Walter Reed Hospital for psychiatric observation and diagnosed for anxiety reaction, acute agitation and hysterical reaction; he was later released for duty.

SUMMARY OF VOLUNTEER TESTING WITH DFP (A6)

A total of 11 subjects was tested with DFP under various test conditions. Five (5) subjects were selected for review, representing approximately 45% of the test group. Selections were based upon the following criteria:

No Treatment (1 subject)
Route: Intramuscular (16.0 µg/kg) 1 subject
Treatment (4 subjects)
Route: Intramuscular (40 - 55 µg/kg) (PAMCl) 3 subjects
Intramuscular (65 µg/kg) (PAMCl + Atropine) 1 subject
(Weak, shaky knees, dizziness, nausea, blurred vision, perspiration).

SUMMARY OF VOLUNTEER TESTING WITH EA 3148 (A7)

A total of 32 subjects was tested under various conditions with EA 3148. Sixteen (16) subjects were selected for review, representing 50% of the test group. Selections were based upon the following criteria:

No Treatment (13 subjects)
Route: Intravenous (0.7 - 1.15 µg/kg) 13 subjects
Treatment (3 subjects)
Route: Intravenous (0.7 µg/kg) (PAMCl) 1 subject
Intravenous (1.15 µg/kg) (Scopolamine) 2 subjects

Subjects with large doses experienced a rapid, severe depression of RBC ChE following agent administration. For example, volunteer #3799 (case #1385), who received 1.15 µg/kg EA 3148, showed 22% of normal RBC ChE values at 15 min after dosing and 0% after 48h; this recovered to 88% of normal at 72d post-exposure.

Of the subjects that received no additional treatment, two subjects who had received the highest dose (1.15 µg/kg) showed toxic signs with 5 to 8 minutes post-exposure. These subjects felt dizzy, weak, tired, sweating, with hands and feet very moist. Within 2 hours post-exposure, these subjects reportedly were resting, eating well and feeling fine. Anorexia, poor sleep, fatigue, unusual dreams, dizziness, euphoria, blurred vision, increased salivation, restlessness are recorded in an Army report summarizing the experience with EA 3148 (CRDL Tech. Memo 2-31). The report also notes one individual whose schizoid personality seemed to be exaggerated by the drug. Four individuals had decrements on the test of numerical facility.

Subjects treated with PAMCl or Scopolamine did not have any severe drop in ChE, and there were no long lasting effects of the drug observed.

SUMMARY OF VOLUNTEER TESTING WITH THA (A9), Tacrine

A total of 15 subjects was tested under various conditions with THA. Five (5) subjects were selected for review, representing approximately 33% of the test group. Selections were based upon the following criteria:

No Treatment (2 subjects)
Route: Intravenous (24 mg, total) 1 subject
Oral (250 mg, total) 1 subject
Treatment (3 subjects)
Route: Oral (192-200 mg, total) (Atropine) 2 subjects
Oral (250 mg, total) (PAMCl+Atropine) 1 subject

The subjects' blood cholinesterase levels were monitored continuously, along with EEG, EKG, respiratory rate and volume with pneumotact, and blood pressure. There were no significant changes in the parameters measured. Untreated subjects were asymptomatic at the dose levels given, and even though there was some drop in ChE (28% of normal) in plasma, the RBC ChE was unaffected. Subjects treated with atropine alone, or with PAMCl, responded well to relief of severe parasympathomimetic reactions. No residual effects of the drug reportedly were observed in any of the subjects tested.

SUMMARY OF VOLUNTEER TESTING WITH PHYSOSTIGMINE (A10)

A total of 138 subjects was tested with physostigmine and 22 were selected for review, representing approximately 16% of the test group. The criteria for selection was based upon the following: Dose, Route, Frequency and Treatment.

No Treatment (6 subjects)
Route: Intramuscular, single dose (28.0 µg/kg) 2 subjects
Intramuscular, multiple dose (15-30 µg/kg) 4 subjects
Treatment (16 subjects)
Route: Physostigmine in single (45 µg/kg) and multiple doses over 1 to 2 days was administered intramuscularly in subjects that had been previously dosed with a variety of drugs such as:
BZ (7.4 - 14.5 µg/kg) aerosol inhalation, 2 subjects
3834 (2.0 mg) percutaneous, 1 subject
Atropine (125 µg/kg) intramuscular, 3 subjects
Prolixin (15.0 - 23.0 µg/kg) intramuscular, 6 subjects
302668 (10.0 µg/kg) intravenous, 1 subject
302196 (75.6 µg/kg) oral, 1 subject
TAB (90 mg total) intramuscular, 1 subject

Pretreatment with methyl scopolamine (1.0 mg) 1 subject
Only 2 subjects (A10J) and (A10K) who received doses of BZ and were subsequently treated with physostigmine, showed any prolonged central effects (hallucinations, disorientation, confusion) lasting 4 to 6 days post-exposure. Both subjects were asymptomatic and appeared normal when discharged from test. One subject (A10-0) was exposed to Prolixin (23.0 µg/kg) and then treated with multiple doses (1.0 mg x 7 doses) over a 2-day period, intramuscularly: At 27 hours post-exposure, the subject complained of blurred vision, and facial expression was mask-like, tongue "thick" and jaws open. By 28 hours post-exposure, subject was having an oculo-gyric crisis, mouth to one side, and left foot tremulous and turned inward.

Subject was further treated with Cogentin, intravenously (1.0 mg and 1.3 mg), in two doses. Within 30 minutes post-treatment, subject was relaxed and dozing and, by 31 hours post-exposure, the subject appeared normal. At 50 hours post-exposure, the subject was asymptomatic and was discharged at 73 hours post-exposure with no complaints.

SUMMARY OF VOLUNTEER TESTING WITH PROSTIGMINE (A11)

A total of 27 subjects was tested with prostigmine as a part of the esophageal motility studies. Seven (7) subjects were selected for review, representing approximately 26% of the test group. Selections were based upon the following criteria:

No Treatment (1 subject)
Route: Intramuscular (0.5 mg) 1 subject
Treatment (6 subjects)
Route: Intramuscular (1.5 mg) (Atropine/PAMCl) 6 subjects

The esophageal motility studies (EPP) were performed by the subjects before and after administration of the drug. ChE levels were also determined in many of the subjects. One subject developed severe cramps in the abdomen, necessitating termination of the study (A11B). All other subjects reportedly tolerated all procedures very well.

SUMMARY OF VOLUNTEER TESTING WITH HEXAFLUORENIUM (Mylaxin^R) (A12)

A total of 11 subjects was tested with Mylaxin as a part of an esophageal test. 11 Subjects were selected for review, representing 100% of the test group. Selections were based upon the following criteria.

No Treatment (11 subjects)
Route: Intravenous (0.4 mg/kg) 11 subjects

The majority of subjects tested experienced a sensation of "warmness" in the stomach and nausea very shortly (2-10 minutes) after receiving the drug, followed by vomiting within 5-15 minutes. The plasma ChE levels in all subjects were depressed while RBC ChE levels were not significantly affected. Esophageal motility studies disclosed marked spasm of the lower 2/3 during deglutition. This coincided with depression of the plasma ChE. There were no prolonged or long-lasting effects reported in any of these subjects as a result of this drug. All subjects reportedly had recovered within 24 hours post-exposure.

SUMMARY OF VOLUNTEER TESTING WITH MALATHION (A14)

A total of 10 subjects was tested with 1.1% malathion powder dusted over the entire body for up to 10 days. Five (5) subjects were selected for review, representing 50% of the test group. All of these subjects reportedly were asymptomatic and developed no signs of intoxication. One subject (A14A), showed low RBCChE which could not be ascribed to laboratory error; however, there were no other significant drops in this subject's ChE levels. All other subjects showed no significant decreases in cholinesterase.

SUMMARY OF VOLUNTEER TESTING WITH METHACHOLINE (A20)

A total of 9 subjects was tested with mecholyl alone or with EA 1729. Several of these subjects also received other drugs such as neostigmine, urecholine, PAMCl, epinephrine by various routes. Three (3) subjects were selected for review, representing 33% of the test group.

Although these subjects were tested or treated with several of these drugs, as well as mecholyl, their vital signs reportedly returned to normal and they were all in good condition by the end of the testing period. There were no prolonged or delayed effects from this drug reported in these subjects.

SUMMARY OF VOLUNTEER TESTING WITH URECHOLINE (A21)

A total of 15 subjects was tested with urecholine under various conditions, under the (EPP). Five (5) subjects were selected for review, representing 33% of the test group. Selections were based upon the following criteria:

	<u>No Treatment (2 subjects)</u>
Route:	Subcutaneous (5.0 mg) (single dose) 1 subject
	Subcutaneous (5.0 mg) (multiple dose) 1 subject
	<u>Treatment of Multiple Compounds (3 subjects)</u>
Route:	Subcutaneous (5.0 mg) (PAMCl+Prostigmine) 1 subject
	Subcutaneous (5.0 mg) (Prostigmine+Curare+PAMCl) 1 subject
	Subcutaneous (5.0 mg) (Tubocurare+Prostigmine+Atropine)
	1 subject

EPP were performed before and after administration of the drug. ChE was also monitored in some cases. All subjects tolerated these procedures very well, and there were reportedly no untoward effects of the drugs in any of the subjects tested.

APPENDIX F

EXCERPTS FROM

BZ DATA

Contract DA-18-108-405-CML-826

Hazelton Laboratories
Falls Church, Virginia

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APPENDIX G

SUMMARY OF ACUTE- AND CHRONIC-TOXICITY DATA IN VARIOUS MAMMALS WITH THE ANTICHOLINERGIC TEST AGENTS (DITRAN, BENACTYZINE, EA 2545, EA 3167, EA 3443, EA 3392, EA 3580, EA 3834, and 226,086)

by

Leo G. Abood, Ph.D.*

Studies were carried out by Lakeside Laboratories (now incorporated into Merrill Dow Pharmaceuticals, Inc.) in rodents and dogs. The results can be summarized as follows.

TOXICITY

Acute. The intravenous LD₅₀ for male rats was 28 mg/kg, and that for male mice was 45 mg/kg.

Chronic. At 8 mg/kg (twice a day) for 8 wk, there was no change in weight of rats; at 300 mg/kg, there was a 12% decrease in weight, compared with controls. In dogs there was no significant change at 3.75 mg/kg.

BLOOD (RATS)

At 300 mg/kg, hemoglobin was reduced by 8%, compared with controls. Sedimentation rates increased by a factor of 3. There was a slight shift in the lymphocyte-to-neutrophil ratio. Leukocyte count increased by about 50% over 8 wk. Terminal red cell count was reduced by 21%.

HISTOPATHOLOGY

At 300 mg/kg, very few abnormalities were observed in rats. An increase in heart weight occurred in some rats; other organ weights remained normal. There were no histopathologic signs at 300 mg/kg; occasional slight changes were due to infection.

In dogs, there were no histopathologic signs attributable to Ditrane at 37.5 mg/kg (highest dose). The blood picture was essentially unchanged.

OTHER DRUGS

Acute- and chronic-toxicity tests were performed by various qualified laboratories at Edgewood laboratories on the various test agents used in the Edgewood study in human volunteers. Acute toxicity was usually studied in mice, rats, cats, dogs, and monkeys to determine the LD₅₀ and to observe pharmacologic effects, such as autonomic changes, changes in motor activity, ataxia, prostration, and convulsions. All the compounds were similar in the spectrum of their pharmacologic effects and differed mainly in relative potency and

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LD₅₀. For the most part, their LD₅₀s corresponded to central nervous system potency.

Chronic toxicity was usually studied over a period of a month, with single daily injections of at least 3 doses of the test agent, the largest dose being near the LD₅₀. Essentially no gross pathologic effects were noted at any of the doses. Occasionally, at nearly lethal doses, slight changes were observed in the weights of some organs--liver, heart, spleen, and thymus. There were also slight changes in blood cells (e.g., increased leukocyte counts) and the Paneth cells of the intestine (decreased counts).

CONCLUSION

On the basis of the acute- and chronic-toxicity data, the agents were deemed to be safe for human trials, particularly for acute studies of the type performed at Edgewood. It should be noted, however, that no attempt was made to observe any long-term behavioral effects, after either acute or chronic drug administration. Behavioral studies are difficult to perform, requiring a battery of complex operant and psychophysical measuring devices. Even with behavioral testing, it is questionable whether subtle long-range behavioral changes could be detected in animals, particularly in acute studies.

APPENDIX H

EVALUATION OF DATA FROM SHORT-TERM GENETIC TESTING OF ANTICHOLINERGIC CHEMICALS

by

Virginia C. Dunkel, Ph.D.*

A number of short-term tests can be used to determine the genotoxic potential of chemicals. These tests use both prokaryotic and eukaryotic cells and measure such end points as gene mutations, chromosomal aberrations, and interactions with critical macromolecules. It is widely recognized that no test can detect all genotoxic compounds, and multiple end points are required to provide a reliable assessment of genotoxicity. Information from several tests can be combined to reveal two important toxic effects: carcinogenesis and mutagenesis.

There are reports of studies in which short-term tests have been used to determine the genotoxic potential of the anticholinergic compounds 3-quinuclidinyl benzilate (BZ), atropine, and scopolamine. The objective of this discussion is to evaluate the data available on these compounds, to determine whether they are genotoxic. Such information would aid in the overall assessment of the potential of these drugs to produce long-term adverse health effects.

3-QUINUCLIDINYL BENZILATE

In the reported studies of (BZ), the test systems used have had three different end points: point mutations, chromosomal aberrations, and dominant-lethal effects.

In the point-mutation studies (1), *Saccharomyces cerevisiae* was used as the target cell, and the effects of the chemical were tested in a direct in vitro assay without metabolic activation and in a host-mediated modification. In the direct reverse-mutation assay, there were increases by a factor of 2-6 in the number of His⁺ revertants and by a factor of 2-7 in the number of trp⁺ revertants over the spontaneous frequencies. In contrast, there were no reported increases in the number of His⁺ or trp⁺ revertants in the host-mediated assay.

Cytogenetic analyses for chromosomal aberrations were carried out on cells from mice (1) and Chinese hamsters (12) treated with BZ and on human peripheral lymphocytes (1) treated in vitro. It was reported that BZ did not induce translocations and did not increase the number of abnormal metaphases in spermatocytes from male mice.

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With bone marrow cells from treated mice, an increase in the frequency of abnormal metaphases was reported, but there is no information on the type of abnormality observed. Similarly, in the studies with human peripheral lymphocytes, "aberrant cells" were observed at a single concentration of BZ (10^{-4} M), but again the type of aberration was not identified. Lower concentrations had showed no effects, and higher concentrations were reported to be toxic. In the studies with bone marrow cells from BZ-treated Chinese hamsters, Sram (2) reported that there was a dose-related increase in the number of gaps and that the frequency of breaks was increased, but not dose-related. That the occurrence of gaps is not a useful measure of the clastogenic potential of a chemical has been substantiated in other studies (3). In addition, although it was concluded that the frequency of breaks observed was not dose-related, it appears from the data that there were increasing numbers with increasing dose, which plateaued and then began to decrease at the highest doses tested.

The final system used in the evaluation of BZ was the dominant-lethal assay (1). The only effect observed in these studies was a decrease in the fertility of the males at the highest dose tested. Such an effect is indicative only of the toxic effects of the test compound.

Overall, at most a marginal response might be indicated by the results of the point-mutation assays with Saccharomyces cerevisiae and the possible presence of cytogenic effects--breaks in Chinese hamster bone marrow cells. The significance of these effects, however, is open to question, and further studies are required to confirm or negate the original observations. Since these studies were reported, a substantial amount of information has been obtained on the predictive capacity of short-term tests, and other assays, such as of gene mutation in bacteria and mammalian cells and of unscheduled DNA synthesis, might provide a better base of information for reaching a conclusion about the potential genotoxicity of BZ.

SCOPOLAMINE

The studies reported on scopolamine are also limited and inconclusive, even though the capacity to induce DNA damage and point mutations in bacteria and chromosomal aberrations in mammalian cells has been tested.

No effects were observed in either the DNA-damage assay (4) or the point-mutation assay in *Salmonella* (5), but the data available on neither of these systems can be considered sufficient for evaluation. In the DNA-damage assay, the compound was treated at only two doses and only in the absence of an exogenous metabolic activating system. The testing in *Salmonella typhimurium* (the *Salmonella* microsome (test), although conducted both with and without metabolic activation, was at a single concentration, and only two test strains were used. In testing of a compound for its ability to induce mutations in this assay, it is necessary to use a range of concentrations and at least four of the five test strains (6).

In the studies carried out to determine whether scopolamine had the capacity to induce chromosomal aberrations, three different types of mammalian cells were used: HeLa and BSC-K cell lines and human peripheral leukocytes (7). No effects were observed in either the human leukocytes or the BSC-K cells, but chromatid aberrations were observed in HeLa cells at a final 1% concentration of the compound. The aberrations were gaps and breaks, but there was no evidence of exchange figures, such as translocations. As indicated previously, the presence of gaps is not a significant measure of clastogenic potential, and the presence of breaks in a single cell line does not establish the mutagenic potential of a chemical.

The available data on scopolamine are insufficient, and a conclusion cannot be drawn as to whether this chemical is potentially genotoxic.

ATROPINE

As is the case with BZ and scopolamine, reports are available on the testing of atropine for its capacity to induce DNA damage and point mutations in bacteria and chromosomal aberrations in mammalian cells. With one exception, these studies are deficient in one respect or another, and no definitive conclusion can be drawn from them. The problems include lack of incorporation of a metabolic activation system in assays in which the target cell does not have full metabolic capabilities (4,8); lack of a range of doses up to and including one at which toxic effects are observed, that reaches the level of solubility, or that is recognized as sufficient for testing (4,9); lack of specification of the dose used (10); and inclusion of a test method, induction of chromosomal aberrations in grasshopper spermatocytes (11), with which experience in genetic toxicology is limited.

In the single study in which there is adequate information, McCann *et al.* (12) reported that atropine does not induce point mutations in the Salmonella/microsome assay. The compound was tested at up to 5,000 µg/plate, both with and without metabolic activation, in test strains that can reveal both base-pair and frameshift mutations. This negative result, however, does not in itself provide sufficient information to justify a conclusion about the genotoxic potential of the compound.

CONCLUSIONS

The lack of adequate data, including test results, and limitations in the test systems used preclude a definitive assessment of the genotoxic potential of BZ, scopolamine, and atropine. Only through further studies can a conclusion be reached about the genotoxic potential of these chemicals.

REFERENCES

1. Sram, R.J., Kuceroval, M., and Bardode, Z. 1975: Mutagenic activity of 3-chinuclidylbenzilate. Act. Nerv. Super., 17:252-253.
2. Sram, R.J. 1975: Cytogenic analysis of 3-chinuclidylbenzilate, 3-chinuclidinol and benactyzine in bone marrow of Chinese hamster. Act. Nerv. Super., 17:253-254.
3. Legator, M.S. Palmer, K.A., and Adler, I-D. 1973: A collaborative study of in vivo cytogenetic analysis. I. Interpretation of slide preparations. Toxicol. and Applied. Pharm., 24:337-350.
4. Fluck, E.R., Poirier, L.A. and Ruelius, Hans, W. 1976: Evaluation of a DNA polymerase-deficient mutant of E. Coli for the rapid detection of carcinogens. Chem. Biol. Interactions, 15:219-231.
5. Waskell, L. 1978: A study of the mutagenicity of anesthetics and their metabolites. Mut. Res., 57:141-153.
6. deSerres, F.J. and Shelby, M.D. 1979: Recommendations on data production and analysis using the Salmonella/microsome assay. Environ. Mutagen., 1:87-92.
7. Vrba, M. 1967: Chromosomenaberrationen, hervorgerufen durch scopolaminum hydrobromatum. Humangenetick, 4:371-373.
8. Ishidate, Jr., M., Hayashi, M., Sawada, M., Matsuoka, A., Yoshikawa, K., Ono, M., and Nakadate, M. 1978: Cytotoxicity test on medical drugs-chromosome aberration tests with Chinese hamster cells in vitro. Eisei Shikenyo Hokoku, 96:55-61.
9. Commoner, B. 1976: Reliability of bacterial mutagenesis techniqueto distinguish carcinogenic and non-carcinogenic chemicals. UISNTIS PB Report; EPA-600/1-76-022.
10. Minnich, V., Smith, M.E., Thompson, D. and Kornfeld, S. 1976: Detection of mutagenic activity in human urine using mutant strains of Salmonella typhimurium. Cancer, 38:1253-1258.
11. Saha, A.K., and Chattopadhyay, S.C. 1977: Studies on the effect of atropine in the production of chromosome anomalies in spermatocytic chromosomes of Indian acridid grasshopper. Chromosome Information Service, No. 22 pp 3-5.
12. McCann, J., Choi, E., Yamasaki, E., and Ames, B.N. 1975: Detection of chemical carcinogens as mutagens in the Salmonella/microsome test; Assay of 300 chemicals. Proc. Natl. Acad. Sci. USA, 72:5135-5139.

APPENDIX I

DIGEST REPORT--ANTICHOLINERGIC CHEMICALS

Introduction

Esters of Tropic Acid

- Studies in Animals
- Human Data from General Literature
- Human Data from Edgewood Arsenal
- Summary

Esters of Benzilic Acid

- Introduction
- Data from Experiments with Animals
- 3-Quinuclidinyl Benzilate (BZ or EA 2277)
- Diethylaminoethyl Benzilate
- Effects on Man
- Human Data from Edgewood Arsenal
- Summary

Esters of Phenylcyclopentylglycolic Acid

- N-Methyl-4-piperidinyl-(phenylcyclopentyl)-glycolate (EA 3443)
- Ditran (CS 4297)
- 3-Quinuclidinyl-(phenylcyclopentyl)-glycolate (EA 3167)
- L-2- α -Tropinyl-L-(phenylcyclopentyl)-glycolate
- cis-2-Methyl-3-quinuclidinyl-(phenylcyclopentyl)-glycolate
(301,060)

Esters of Phenylisopropylglycolic Acid

- N-Methyl-4-piperidinyl-(phenylisopropyl)-glycolate (EA 3834)
- 4-(1-Methyl-1,1,2,3,6-tetrahydropyridinyl)-phenylisopropyl-
glycolate (302,668)

Esters of 1-Propynylcyclopentylglycolic Acid

- N-Methyl-4-piperidyl-(phenylcyclobutyl)-glycolate (EA 3580)

Miscellaneous Esters

- 302,282
- 302,537
- WIN 2299

Nonester Anticholinergic Compounds

- Benzetimide (CAS 14051-33-3)
- Mepiperphenidol

"TAB" Mixture

Discussion

Note: The chemical nomenclature may differ from that given in the master file. The EA or other number serves for identification of the compound in the master file.

APPENDIX I

DIGEST REPORT ANTICHOLINERGIC CHEMICALS

by
J. Henry Wills, Ph.D.

INTRODUCTION

The Board on Toxicology and Environmental Health Hazards of the National Research Council provided 58 reports that are related to the administration of anticholinergic compounds to human volunteers by personnel and contractors of the Chemical Corps Medical Laboratories at Army Chemical Center, Md., and its successor organizations at Aberdeen Proving Ground, Edgewood, Maryland. Those reports recorded studies of the responses of humans, mostly men, to 19 individual substances and to a mixture of atropine, benactyzine, and the oxime, trimedoxime (TMB-4). Two of the 19, benzetimide (Dioxotrine) and mepiperphenidol (Darstine), are N-alkylated derivatives of piperidine, but the other 17 are esters. Anticholinergic substances not mentioned in these reports, but said to have been administered also to volunteers, are N-methyltropinylphenylcyclopentylglycolate, 3-quinuclidinyl-1-hydroxyphenylcyclopentylacetate, and N-diethylaminoethylphenylcyclopentylcarboxylate.

The 17 esters represented in the 58 reports mentioned above belong to seven series of compounds: esters of tropic acid, of benzylic acid, of phenylisopropylglycolic acid, of phenylcyclopentylglycolic acid, of 3-quinuclidinol, of N-methyl-4-piperidinol, and of N-diethylaminoethanol.

Esters of tropic acid occur in nature and have a comparatively long history of use as drugs. Atropine (DL-hyoscyamine) and its optical isomers, the D- and L-hyoscyamines, and scopolamine (L-hyoscyne) and its optical isomer, D-hyoscyne, have been obtained from such plants as deadly nightshade (Atropa belladonna), jimsonweed (Datura stramonium), henbane (Hyoscyamus niger), horsenettle (Solanum carolinense), and various species of Scopolia. The L isomers of both esters are more potent than the D isomers.

Both esters have peripheral and central anticholinergic activities; i.e., they interfere with the actions of acetylcholine at peripheral neuroeffector junctions on smooth and cardiac muscles and on cells of glands that produce external secretions and at synapses between some neural elements. Scopolamine is especially potent in the last regard, whereas atropine and L-hyoscyamine are especially effective in blocking the action of acetylcholine at peripheral neuroeffector junctions. Even at neuromuscular junctions on skeletal muscles, which atropine usually is considered not to affect significantly, a sufficiently high concentration (10^{-4} M) of this drug in vitro caused a shortening of the duration of end-plate currents (1).

Quaternization of these alkaloids removes much of their ability to penetrate into the central nervous system and to affect its functions. However, the same variation in chemical structure increases the weak ability of these materials to interfere with the action of acetylcholine at the motor end plates of skeletal muscles.

Toxic psychoses and delirium from ingestion of atropine and scopolamine have been known for many centuries; descriptions of the effects of these drugs antedate by long times the recognition of their mechanism of action. For example, henbane was recommended in the Ebers papyrus of about 1550 B.C. for the relief of abdominal distress.

After the first isolation of atropine from plant material in 1832, the actions of this group of alkaloids were identified rapidly. By 1875, Ringer (2) was able to give the following description (excerpted) of the actions of atropine (belladonna):

Belladonna employed either internally or externally checks or even suppresses the secretion of the glands. This at least is true of the mammary, sudoriparous and salivary glands, and possibly of other glands.

Its influence on the secretion of the submaxillary glands has been fully worked out. This gland receives branches from the chorda tympani nerve which is endowed with two sets of fibres, one acting immediately on the cells, the other causing the blood-vessels to dilate, being vaso-inhibitory. Belladonna acts through the nerves distributed to the cells, for after the injection of atropia, if the chorda tympani nerve is irritated, the vessels of the submaxillary gland become distended as usual, but the gland does not secrete. The paralyzing effect of atropia is antidoted by physostigma, for after the injection of physostigma, irritation of the chorda tympani causes the gland to secrete.

Dropped into the eye, applied to the skin in its neighborhood, or taken by the stomach, preparations of belladonna very speedily produce extreme dilatation of the pupil. This is one of the most characteristic effects of belladonna.

A full dose of belladonna produces great dryness of the tongue and roof of the mouth, extending down the pharynx and larynx, inducing consequently some difficulty in swallowing, with hoarseness, and even dry cough; and a large dose will sometimes induce dryness of the Schneiderian membrane, and dryness of the conjunctiva, with much injection.

Belladonna often relieves colic of the intestines; and is especially serviceable in the colic of children.

After a considerable dose of belladonna, the face becomes much flushed, the eye bright, dry, and injected, the pupil dilated, the sight dim and hazy, while the power of accommodation in the eye for

distance is lost. The mind and senses are peculiarly affected. The ideas, at first rapid and connected, become incoherent and extravagant; there is often decided delirium, with pleasing illusions. Sometimes the patient is possessed with constant restlessness, keeps continually moving, and cannot be quieted. A kind of somnambulism is occasionally observed; thus cases are recorded where, under the influence of belladonna, the patient for a long time performs the movements customary to his occupation; thus, it is narrated of a tailor that he sat for hours moving his hands and arms as if sewing and his lips as if talking, but without uttering a word.

The delirium may be furious and dangerous, requiring the patient to be restrained; nay, it is recorded of one poisoned by this drug that so violent did he become that he was ordered to be confined in a mad-house.

The first effect of belladonna on the pulse is to increase its quickness, fullness, and force to the extent even of 50 to 60 beats in the minute. . . . Meuriot is of opinion that belladonna paralyzes the peripheral branches of the vagal nerve, and by this means accelerates the heart's action.

Ringer had this to say about the effects of hyoscyamus (scopolamine):

Thus it produces dryness of the mouth and throat, dilatation of the pupil, presbyopia, lightness and swimming in the head, delirium and hallucinations, a drunken gait, and often a strong desire to fight. Sometimes there is aphonia, and often sleepiness, with oppressive disagreeable dreams. A red rash has been observed after large doses. The pulse at first is much lessened in frequency but soon recovers itself, sometimes becoming even quicker than before the medicine was taken.

Hyoscyamus is generally used to produce sleep when opium disagrees. It has also been employed in neuralgia.

It was only after the role of acetylcholine in the functioning of the autonomic nervous system, in neuroeffector transmission to the various entities innervated by that system and to skeletal muscles (3-11), and in synaptic transmission within some areas of the central and peripheral nervous systems (12-17) had been demonstrated that the proximate mechanism of action of atropine, scopolamine, and other compounds of similar activity could be understood. These compounds seem to attach to the same receptors to which acetylcholine usually attaches itself and thus to interfere with the ability of acetylcholine to produce changes in the properties of cellular membranes.

The actions of atropine and scopolamine to block synaptic transmission within some areas of the central nervous system and to induce coma were applied to the treatment of

mental disease by Forrer (18) and Goldner (19). In 1950, Forrer published an account of a study of 16 schizophrenic patients treated intramuscularly with large doses of atropine sulfate on 3 days of each week for a long period. The doses of atropine sulfate were increased gradually during a course of treatment from 20-32 mg to 212 mg, to maintain the achievement of unconsciousness, with hyperreflexia and development of the Babinski sign, despite a gradually developing tolerance to the drug.

In 1957, Miller *et al.* (20) reviewed the cases of 206 mental patients treated in this general way, including 148 who had been ill for 2 yr or more before treatment. At the time of that report, the technique was to inject atropine sulfate at 3:00 a.m., so that the patient would recover sufficiently from the effect of the drug to be able to eat lunch and take part in organized activities in the hospital during the afternoon and evening of the same day. Four treatments were given each week, instead of the three that had been given initially. The desired state of coma was produced within 30-60 min of intramuscular injection, and spontaneous recovery, with complete clearing of the sensorium, required 6-9 h after the injection. Of 206 patients treated, 115 (55.8%) were considered to have improved moderately or markedly, 60 (29.1%) to be unimproved, and 31 (15.0%) to have improved only slightly. Six months after completion of a course of injections, 115 patients (55.8%) were still improved moderately or markedly.

In a series of about 500 patients treated with large doses of atropine, with about 10,000 individual inductions of coma, there was one death (21). This fatality was attributed to the replacement, without notification of the physician, of the specially trained nursing and technical personnel on the treatment ward with persons who were not thoroughly familiar with the procedures for caring for comatose patients, and particularly the procedures for controlling febrile reactions. The patient died of uncontrolled hyperthermia.

Goldner (19) reported that he had used intramuscular doses of 5-50 mg of scopolamine in treating beneficially an unspecified number of mental patients, but then had switched to atropine because of its greater availability. He reported on a group of 20 patients treated with atropine-toxicity therapy, 13 (65%) of whom were considered to have benefited moderately or markedly. Goldner also gave a limited comparison of the results of electroconvulsive therapy (ECT) and of atropine-toxicity therapy, stating that several patients who had not benefited from ECT improved on substitution of atropine-induced coma.

Wada *et al.* (22) reported on 51 psychoneurotic and psychotic patients treated with intramuscular injections of 10-220 mg of atropine sulfate every other day during a period of 3-7 wk. The average dose was 30-50 mg. The longest period of observation mentioned in the paper was 6 mo. Of the 51 patients, 28 (54.9%) were considered to have improved detectably. The proportion that improved among the psychotic patients (53.3%) was smaller than that among psychopathic and

psychoneurotic ones (66.7%). The greatest alterations in symptoms and signs of the patients' diseases were in psychomotor excitement, hallucination, euphoria, anxiety, compulsion, and delusion. Wada et al. stated that improvement, when it occurred, began with the third to the seventh injection and increased with later injections. If no improvement occurred by the tenth injection, it was not likely to appear. The authors concluded that atropine-toxicity therapy is a relatively safe type of shock therapy, but stated that it was successful in only about 53.3% of their psychotic patients and that its efficacy probably had been overestimated by Schwarz (23). Schwarz had concluded that 25% of 155 cases of psychoneurosis and psychosis had improved markedly with atropine-toxicity therapy and that another 32% had improved moderately.

Miller et al. (20) described the phenomena experienced by patients given large doses of atropine to induce a toxic coma:

The various stages are passed through quietly and comfortably. Neurologically, there are in order: progressive muscular incoordination, decreased pain sensitivity, and hyperreflexia with the development of the Babinski sign. On the psychological side, we observed clouding of the sensorium, disorientation, loss of time-space relationship, distortion of perception by illusions and hallucinations, confusion, and coma which proceeds to but does not go further than the early fourth stage in one and one-half hours after the drug is administered.

Although the sensorium is markedly clouded, there is no cessation of psychological phenomena. Patients undergoing treatment indicate by their actions, and occasionally by word, that psychic processes continue even during the coma. Illusions seem to be experienced, inasmuch as patients attempt to pick up the bedclothing with their fingers and later report that they saw, and attempted to pick up, flowers, bugs, snakes, et cetera. Conversations may be held with absent persons--indicative of an hallucinatory state. With both atropine-induced illusions and hallucinations, highly significant past events in the patient's life seem to provide the major psychic stimuli. Behavior is correlated with psychological phenomena. Circumoral movements, including sucking and illusory "eating" and "smoking," are commonly observed. When a sufficient degree of motor coordination is retained to accomplish the necessary movement, a progressive, coordinated, and purposeful series of events may often be observed. For example, a pantomime of striking a match, lighting a cigarette, and subsequently of smoking that cigarette is not infrequently observed. Affective lability and corresponding rapid alternation of facial expressions suggestive of pain, sadness, querulousness, and euphoria are commonly observed.

This clinical experience indicating that even large and temporarily incapacitating doses of atropine and scopolamine had no persistent deleterious effects on the health of human

beings made it seem that the administration of other, possibly more incapacitating, anticholinergics to human volunteers was not unethical or immoral if two conditions had been fulfilled. These were that a compound to be administered to volunteers had been tested adequately in experimental animals to determine that the probability of its having persistent ill effects on health was small and that the volunteers had been informed as fully as possible of the possible effects of the compound. In the cases of compounds that had been administered to man previously, the effects could be described quite accurately; if the only previous recipients had been experimental animals, the description of possible effects had to be somewhat hypothetical but usually could be reasonably accurate.

Before a discussion of the specific groups of anticholinergic compounds, it may be worth while to have a general view of the procedure of the experiments carried out with human volunteers at Edgewood Arsenal and followed also by contractors. The following description is a composite from two sources: a report from Edgewood Arsenal (24) and a report by a contractor (25).

Test subjects were selected in two stages. Groups of possible volunteers were acquainted with the general nature of the experimental program through a briefing, during which they could ask questions about the general conditions and procedures of the studies. Those who volunteered to take part in the experimental program after this general briefing were given thorough medical examinations, including psychologic or psychiatric assessment. The volunteers considered acceptable for a particular experiment were then given a specific briefing and an opportunity to volunteer to take part in that study.

The studies were carried out in air-conditioned areas from which hazards of accidental injury had been eliminated as thoroughly as possible (e.g., glass panels in doors were replaced with wood, electric outlets and cables shielded, and sharp corners padded). Beds and toilet, messing, and recreational facilities were provided within the research areas. The research was conducted under surveillance of qualified physicians, who administered all injections or oral doses of the test substances. When volunteers were exposed to inhalation of vapors or aerosols, the concentrations of these substances in the air to be breathed by the subjects were established by appropriate personnel. Breathing of the contaminated air by the volunteers was observed and supervised by physicians. After an exposure to an agent, the volunteers were observed continuously for 8-24 h, and then intermittently for up to 14 d. In a few cases, followup studies were made 6 mo or more after the exposure.

ESTERS OF TROPIC ACID

This group contains five of the substances said to have been administered to volunteers, but only three of these are mentioned in the 58 reports related to studies of the effects of anticholinergic compounds on human beings: atropine, scopolamine, and methylscopolammonium. The other two substances in this group that were administered to volunteers are D-hyoscyamine and methylatropinium. Atropine and scopolamine appear, respectively, in 18 and 11 reports, whereas methylscopolammonium salts appear in only three.

Atropine and scopolamine differ only in the basic moiety esterified with tropic acid. Scopolamine and the hyoscines have an oxygen bridge inserted between the two carbon atoms in the dihydropyrrole ring of tropine that are not adjacent to the nitrogen atom, forming scopine, whereas atropine and the hyoscyamines have no such bridge. The facts that atropine is a racemic mixture of D- and L-hyoscyamines, that scopolamine is L-hyoscine, and that the L forms of both hyoscyamine and hyoscine have much greater biologic effectiveness than the D forms mean that atropine sulfate, with only 50% of its active isomer, has only about 60% as much active material per unit of weight as scopolamine hydrobromide, despite the addition of an oxygen atom in the bridge in the latter molecule.

STUDIES IN ANIMALS

Several relatively recent reviews of the actions and potencies of the solanaceous alkaloids are available (26-32). Generally, they give a great deal of information about the medically useful or undesirable actions of the compounds without dealing with their toxicity in any quantitative manner. Table I-1 contains a summary of information on the LD₅₀ doses for experimental animals of the five compounds in the group of esters of tropic acid, collected from a variety of published sources (33-41) and from internal reports of Bristol Laboratories, Lederle Laboratories, Maltby Laboratories, the Squibb Institute for Medical Research, Strassenburgh Laboratories, the Upjohn Company, and Sterling-Winthrop Research Institute.

Albanus (42) studied the effects of subcutaneous injections of 13 anticholinergic compounds on central and peripheral cholinergic activities in the dog, recording such factors as the appearance of ataxia and of what he called "obstinate progression" (i.e., the failure of the animal to pull back after encountering an obstacle in its path), the light reflex of the iris, salivation, and heart rate. The compounds administered to dogs included atropine, scopolamine, and a methylatropinium salt. A dose of atropine at 0.1 mg/kg produced no significant alterations in the dogs, but a dose of 0.2 mg/kg produced ataxia in three of four dogs and increased their heart rate by 76%. A dose of 0.3 mg/kg made five of five dogs ataxic and caused three of five to engage in obstinate

progression. A dose of 0.5 mg/kg rendered seven of seven dogs both ataxic and out of contact with the environment, as indicated by obstinate progression. This dose also abolished the light reflex of the iris, blocked salivation between 45 and 180 min after the dose, and increased heart rate by 89%.

Scopolamine was considerably more active than atropine in this series of experiments, a dose of 0.03 mg/kg producing ataxia in six of six dogs and increasing heart rate by 24%. A dose of scopolamine at 0.05 mg/kg rendered four of four dogs ataxic and caused them to engage in obstinate progression. This dose also resulted in abolition of the light reflex of the iris, paralysis of salivation between 45 and 135 min after the dose, and a 59% increase in heart rate.

The methylatropinium salt was much less effective than either of the native alkaloids. A dose of 2.5 mg/kg produced ataxia in none of four dogs. A dose of 10 mg/kg induced ataxia in five of eight dogs and obstinate progression in three of eight. It abolished both the light reflex of the iris and salivation between 45 min after the dose and the end of the period of observation, at 315 min after the dose. Doubling that dose induced both ataxia and obstinate progression in four of four dogs.

The only study of the subchronic toxicity of members of the group of esters of tropic acid found is one by Boyd and Boyd (43) with atropine. Groups of rabbits were given daily intramuscular injections of atropine sulfate at 44-118 mg/kg for 100 d. The daily dose required to cause death within 100 d was 78 ± 5 mg/kg, whereas that required to cause death within 10 d was 127 ± 9 mg/kg. The Boyds obtained a relationship between the number (y) of daily injections to produce death and the size of the daily dose (x): $\log y = 2.35 - 0.343 \log x$. The principal sublethal effects noticed in the rabbits were (in the approximate order of their appearance): increased intracolonic temperature, decreased drinking, decreased production of urine, and decreased growth.

Three other papers were related to a special type of subchronic toxicity induced by atropine: alteration of cells, embryos, and fetuses exposed to the chemical. Ishidate et al. (44) exposed cultures of fibroblasts from Chinese hamsters to atropine sulfate at 250 ug/ml in saline for 48 h. At the end of the period of incubation, 1% of the cells were polyploid. Of cells examined for chromosomal aberrations after incubation with atropine for 24 h, 1% had gaps and breaks in their chromosomes. These percentages of polyploidy and of chromosomal aberration were only slightly greater than those in cultures to which plain saline had been added. Examination of atropine sulfate for carcinogenicity in animals by the method specified in Survey of Compounds Which Have Been Tested for Carcinogenic Activity (U.S. Public Health Service Publication 149) and for mutagenicity in bacteria yielded no evidence that atropine had either activity.

Butros (45) explanted 21- to 22-h chick blastoderms onto agar-containing substrates with atropine at 40-100 µg/ml for 24 h. Blastoderms exposed at 40-80 µg/ml had slight but dose-related injury to the developing neural tube. Those exposed at 90 µg/ml had arrested development with pycnosis and slight cytolysis of the cells of the brain wall and with somites that were smaller than normal and that had slightly opaque cells. Blastoderms exposed at 100 µg/ml had their growth arrested and their brain walls wrinkled and folded, the cells evincing early cytolysis. In some of these blastoderms, the neural tube was completely cytolized; in others, the neural tube was open at both its ends. Some 85% of the embryos had normal beating heart tubes; some heart primordia were incomplete and had lateral gaps.

Grunfeld and Balazs (46) reported that pregnant rats given daily subcutaneous injections of atropine sulfate at 0.1 or 0.5 mg/kg-d from day 11 to day 18 of pregnancy had low heart rates, hypothermia, and greater than usual slowing of the heart by a dose of pilocarpine for about a week after the end of administration of atropine. The progeny of these rats had tachycardia for 2 wk after birth and mydriasis for a week after their eyes opened. No long-lasting effects on either the dams or the pups were reported.

Scopolamine, added to the culture medium in a Petri dish at 1 mg/plate after incubation with microsomes prepared from livers of rats, produced no change in the cultural characteristics of Salmonella typhimurium strains TA 98 and TA 100 (47). In agreement with this finding of nonmutagenicity of scopolamine for bacteria, a study by Vrba (48) with HeLa cells, human leukocytes, and monkey renal cells revealed that scopolamine hydrobromide at a final concentration of 1% in contact with these cells induced aberrations of the chromatids in the HeLa cells, but not in the monkey renal cells or the human leukocytes. Vrba concluded that changes in chromatids in HeLa cells do not prove that a chemical is mutagenic in man.

Campbell and Ramirez (49) gave three pregnant rats daily intraperitoneal injections of scopolamine at 1.2 mg/kg. Three other groups of two pregnant rats each were given nothing other than the usual food and water or daily intraperitoneal injections of physiologic saline or scopolamine at 2.4 mg/kg. All experimental procedures began on the tenth day of pregnancy and were continued until parturition. Some of the offspring of these dams, when 120 d old, were deprived of water for 18 h and then placed in a conflict situation in which they were exposed to continuous electric shock of variable voltage whenever they tried to drink. The voltage required to inhibit drinking was recorded. Offspring of the three dams that had received daily doses of scopolamine at 1.2 mg/kg were

said to have tolerated electric shock better than those of dogs under either of the other regimens. No data were presented.

Lavallee (50) collected screening data on scopolamine with dogs and monkeys for comparison with the data from experiments with human volunteers performed at or for Edgewood Arsenal. In dogs, the incapacitating dose of scopolamine had been estimated to be less than 100 mg/kg. In monkeys, with only two animals, only a single dose of scopolamine had been used. The incapacitating dose was clearly above that dose, which had been 32 μ g/kg. In man, the incapacitating dose in 50% of the subjects was said to be about 10 μ g/kg. The agreement between the values for man and for the experimental animals was not good. Indeed, when a series of seven compounds was examined for comparative effectiveness in man and in dogs and monkeys, the comparative grading in man did not agree with those in the other animals.

In addition to the more or less standard actions of anticholinergic compounds due to their ability to interfere with the actions of acetylcholine on muscarinic receptors (resulting in acceleration of the heart; relaxation of smooth muscles in bronchi and bronchioles, intestinal tract, urinary tract, iris and ciliary body of the eye, bile ducts and gall bladder, and some cutaneous blood vessels; and decrease or abolition of secretion by glands of the gastrointestinal tract and skin), these substances may have pronounced effects on the central nervous system, as has been mentioned earlier. A large amount of research with experimental animals has been directed toward elucidation of the basic mechanisms involved in these actions.

Much of the research has been oriented around one of the basic tenets of pharmacology and toxicology: that a chemical entity affects particular structures in the body because they contain particular aggregates and arrangements of molecules and atoms that establish a special affinity between the structures and the chemical (51). As a corollary, the particular aggregate and arrangement of atoms in a molecule of a chemical entity may condition its affinity for a given site, or receptor. Thus, isomeric forms of a molecule may have different affinities for a given receptor, as has been found to be true for the D and L isomers of hyoscyamine and hyoscine (38). Also, fairly small differences between two molecules may make extensive differences in their abilities to react with particular sites in the body. For example, Graham and Lazarus (34) found that methylatropinium nitrate had approximately the same activity as atropine sulfate in preventing cholinergic stimulation of isolated rabbit intestine, but that it was some 3 times as lethal to mice after intraperitoneal injection. They also found that methylatropinium nitrate had a more pronounced inhibitory effect on the rabbit cardiovascular system than atropine sulfate; this

difference between the two compounds certainly contributed to, and may explain, the greater lethality of methylatropinium nitrate. There are indications that quaternization of atropine did not alter extensively its affinity for the receptor on intestinal smooth muscle, but did increase the affinity for receptors on components of the cardiovascular system and perhaps other structures.

Schallek and Smith (52) reported that intravenous injection of atropine at 0.01 mg/kg into dogs anesthetized with thiopental had no detectable effect on their EEGs or their cardiovascular systems. A dose of 0.1 mg/kg increased the predominant frequency in the EEGs of three of five dogs and induced tachycardia in two of five. A dose of 1.0 mg/kg decreased the predominant frequency of the EEGs in four of five dogs and caused hypotension in one of five. Still higher doses increased these actions and also induced bradycardia. Similar effects by atropine and scopolamine in curarized cats and monkeys had been reported previously (53, 54), and stimulation followed by depression by atropine and scopolamine of maze-running by rats had been seen by Macht (55).

In 1963, Zvirblis and Kondritzer (56) found that mitochondria isolated from 0.2 g of brain of young adult white rats adsorbed between 8.4 and 12.6% of the [14 C]atropine in 4 ml of atropine solutions with concentrations of 0.063-0.500 μ g/ml. When the mitochondria isolated from 0.4 g of brain were immersed in the same concentrations of atropine, the adsorption was between 8.0 and 13.5%. If the lower of the last two values is erroneous (it was obtained with the second lowest concentration of atropine) and the correct value should be about 13.3% between the values for the lowest and the next higher concentrations, the range of adsorption by the larger amount of mitochondria would be 11.4-13.5%. Even with substitution of the hypothetical value in the second series, increasing by 100% the mass of brain substance represented by the isolated mitochondria seems to have increased the adsorption of atropine by no more than a mean of about 13%. Aggregation of mitochondria in the higher concentration of these organelles may have reduced the effective surface for adsorption of atropine. In these experiments, Zvirblis and Kondritzer found also that 3-quinuclidinyl benzilate was adsorbed by mitochondria 2.6-3.3 times as avidly as was atropine.

In 1973, Farrow and O'Brien (57) performed a study somewhat similar to that of Zvirblis and Kondritzer, using atropine and muscarone as the adsorbates and various fractions isolated from a homogenate of rat brain as the adsorbents. They found that both adsorbates--but not decamethonium, dimethyltubocurarine, or nicotine--were bound reversibly on mitochondria in a crude fraction. Muscarone was bound to the extent of only about 0.37 times the binding of atropine. Atropine bound especially to fractions that consisted of presynaptic and postsynaptic

membranes, membranes of uncertain origin, microsomes, and synaptosomes. It bound also to homogenates of liver and to mitochondria prepared from liver, but not to homogenates of lung or kidney. Muscarone had a similar pattern of binding to various fractions, except that it did not bind to mitochondria from liver.

Hiley and Burgen (58) used [^3H]propylbenzilylcholine mustard as a model muscarinic agonist to examine the concentrations of muscarinic receptor in various regions of dog brain. Synaptosomes and membranes prepared from the head of the caudate nucleus, the precal gyrus, the uncinate gyrus, the suprasylvian gyrus, the lingual and suprasplenic gyri, and the anterior sylvian gyrus had the largest uptakes of the model agonist, whereas those prepared from the dorsal and ventral halves of the lumbar spinal cord, the subcortical white matter, the pes pedunculi, the corpus callosum, and the cerebellar cortex had the smallest uptakes.

Hiley and Burgen (58) measured also the choline acetylase and acetylcholinesterase activities of selected areas of the brain, in an attempt to determine whether the concentration of muscarinic receptors in a location was related to either of these enzyme activities. The amount of the labeled propylbenzilylcholine mustard bound was not well correlated with either the choline acetylase or the acetylcholinesterase activity; there was a modest correlation between binding of the model agonist by synaptosomes and membranes prepared from nine areas of brain and the choline acetylase activities of the same areas, but it was far from precise.

Burgen *et al.* (59) determined that hyoscine was 10 times as active as atropine in vitro in reducing the uptake of [^3H]propylbenzilylcholine mustard by synaptosomes from rat cerebral cortex. A methylatropinium salt was only slightly less active than atropine, but had a very flat dose-action curve: an increase in its concentration by a factor of 10 increased the percent change in uptake of the propylbenzilylcholine mustard only from 64% to 67%. These investigators also reported that the muscarinic receptor in cerebral cortices of the mouse, the guinea pig, the dog, the pig, and the macaque behaved very similarly to that in the rat.

In 1974, the finding of Zvirblis and Kondritzer (56) of a particularly large affinity of the muscarinic receptors in brain for 3-quinuclidinyl benzilate was applied to the assay of the quantity of muscarinic receptor in a sample of tissue (60, 61). Intramuscular injection of atropine 30 min before intravenous injection of [^3H]3-quinuclidinyl benzilate decreased the binding of the quinuclidinyl benzilate in various regions of the brain. Although the corpus striatum had the highest uptake of the labeled substance, the cerebral cortex was not far behind. The percent decreases by atropine in the uptake of the ligand were approximately equal for the corpus striatum and the cerebral cortex. The hippocampus,

which came next in magnitude of benzilate binding, was also next in magnitude of the change in binding of the benzilate caused by atropine. Indeed, in the six-membered series of corpus striatum, cerebral cortex, hippocampus, hypothalamus, pons-medulla oblongata, and cerebellum, there was complete agreement in rank order for benzilate binding and reduction by atropine of benzilate binding. Administration to rats of mecamylamine, phenobarbital, or L-dihydroxyphenylalanine before intravenous injection of [³H]3-quinuclidinyl benzilate had no effect on benzilate binding in the six areas of the brain.

In these experiments, the highest concentration of labeled benzilate bound to protein was in the microsomal fraction of a homogenate of whole brain, and the next highest was in the mitochondrial fraction, the protein of the microsomal fraction binding nearly 2.5 times as much of the label as that of the mitochondrial fraction. The protein of the nuclear fraction bound only about one-twelfth as much of the labeled benzilate as that of the microsomal fraction and about one-fifth as much as that of the mitochondrial fraction. The muscarinic receptor substance(s) in the rat brain appears to be fairly generally distributed in membranes of subcellular components and to be localized especially in the corpus striatum, the cerebral cortex, and the hippocampus, the last of these three binding nearly 3 times as high a concentration of labeled benzilate as the next most active structure in such binding, the hypothalamus.

Because both muscarinic anticholinergic agonistic drugs (scopolamine, isopropamide, and atropine) and muscarinic agonistic drugs (oxotremorine, acetylcholine, methacholine), but not nicotinic anticholinergic drugs (mecamylamine, hexamethonium, and D-tubocurarine) or nicotinic agonistic drugs (dimethylphenylpiperazinium and nicotine), strongly inhibited the binding of labeled benzilate by homogenates of rat brain, binding of this compound was proposed as an indicator of the presence of muscarinic receptors in particular brain structures. In the nine-membered series of corpus striatum, cerebral cortex, hippocampus, superior-inferior colliculi, pons-midbrain, thalamus, hypothalamus, cervical cord-medulla oblongata, and cerebellar cortex, Yamamura and Snyder (61) found, as had Hiley and Burgen (58) with binding of labeled propylbenzilylcholine mustard, that binding of labeled benzilate was not well correlated with either the choline acetylase or the cholinesterase activity of the particular region, but was slightly better correlated with choline acetylase activity than with that of cholinesterase. Farrow and O'Brien (57) had concluded that binding of labeled atropine to various subcellular components of the rat brain could not be accounted for by binding to acetylcholinesterase.

Farrow and O'Brien (57) had found also that scopolamine was a much more potent inhibitor of the binding of labeled atropine to synaptic membranes than a

variety of nicotinic anticholinergic and agonistic drugs; the next most active inhibitor used by these investigators had been carbamylcholine chloride, found by Yamamura and Snyder (56) to be less effective than acetylcholine and methacholine in reducing binding of labeled 3-quinuclidinyl benzilate.

In later work, Snyder's group (62,63) used autoradiography to determine more precisely the locations of binding of [^3H]3-quinuclidinyl benzilate within the various regions studied earlier. In the cerebral cortex, the most active areas of benzilate binding were the occipital cortex and the cortex of the cingulate gyrus. The cortex in the piriform area was less active in this regard, and that of the frontal pole still less so. In the hippocampus, three strata of the archipallium were found to have approximately equal abilities to bind 3-quinuclidinyl benzilate, but the white matter of the alveus had much less activity of this sort. The striate body also had high binding activity for this ligand, which was particularly striking in the autoradiographs because of the comparatively low binding activity of the adjoining globus pallidus. Thalamic and hypothalamic nuclei had low binding activities for 3-quinuclidinyl benzilate similar to those of the inferior and superior colliculi. Still lower in activity in binding this ligand were cerebellar cortex, medulla oblongata-pons, and the cerebral peduncles. Nerve tracts--e.g., the optic chiasma, the cervical spinal cord, and the corona radiata--had the lowest binding activities evaluated by Snyder et al.

On the basis of the research outlined in the preceding paragraph, one would expect anticholinergic compounds to produce changes in affect and motor and autonomic regulatory activities. Krieger (64) has found that a dose of atropine that was sufficient to block the circadian increase in the plasma concentration of 17-hydroxycorticosteroids during the night did not prevent stimulation of secretion of 17-hydroxycorticosteroids by the adrenal cortex after administration of ACTH or of stressors, such as insulin and piromen. Thus, the effect of atropine seems to have been exerted not on the adrenal cortex, but rather on the pituitary, the controller of activity by the adrenal cortex through modifiable release of ACTH.

Paton and Rang (65), building on indications in previous papers (66-69) that atropine antagonized the action of acetylcholine in tissues by combining reversibly with specific receptors, found evidence of the existence, in the longitudinal muscle of the small intestine of the guinea pig, of two binding sites for atropine with different capacities for it. The equilibrium constant for binding atropine amounted to slightly more than 5.01×10^{-7} M, or about 1,180 pmol/g of muscle. For methylatropinium salts, binding seemed to be to one site with reasonably definite binding properties and to an ill-defined series of other sites with much higher